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# Evaluating atmospheric pressure cold plasma decontamination techniques for packaging materials: a systematic review and meta-analysis

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Decontaminating food packaging surfaces is a crucial step in the food processing industry to ensure the quality and safety of the product. Decontamination is intended as a procedure aimed to reduce the microbial load present on contaminated packaging to a safe level. Several techniques are traditionally employed, but the industry is seeking innovative methods that could offer economic and environmental benefits. Cold plasma is emerging as a promising solution among the range of possibilities. The present review aims to assess the effectiveness of plasma-assisted systems for decontaminating packaging materials. A systematic collection of inherent records was carried out, and the study outcomes were extracted using the protocol for meta-analysis. The synthesis of the results demonstrates the efficacy of this sanitation technique, since the average logarithmic reduction of the pathogen charge on the packaging was above 4. This outcome is promising since it aligns with standard requirements for traditionally employed antiseptics. Future research should focus on the optimization of processes from the perspective of industrial applications.

KEYWORDS

**cold plasma applications, packaging, decontamination (cleaning), microorganisms, plasma decontamination, atmospheric pressure, foodborne pathogens, food industry**

# 1 Introduction

## 1.1 Foodborne diseases

Foodborne diseases, caused by contaminated food contact or ingestion, pose a significant global health concern. Contamination with bacteria, viruses, parasites, or chemicals can result in various illnesses, with over 200 identified, predominantly affecting the gastrointestinal tract but also leading to neurological, gynecological, and immunological issues [1]. Annually, nearly one in 10 individuals worldwide suffer from foodborne illnesses, resulting in over 420,000 deaths. While foodborne diseases affect all countries, low- and middle-income nations bear a disproportionate burden. Factors such as poverty, international trade, longer food chains, urbanisation, climate change, migration, and increased travel exacerbate these challenges, increasing the risk of contamination and the spread of infections across borders. In the European Union alone, over 5,000 foodborne outbreaks are reported annually, causing approximately 45,000 cases [2]. In 2022, foodborne outbreaks increased significantly compared to the previous year, highlighting the need for stringent hygiene standards and HACCP (Hazard analysis and critical control points) protocols throughout the food production chain to mitigate contamination risks and safeguard consumers.

## 1.2 Food packaging

According to the Food Packaging Forum [3], the most used packaging materials and food contact surfaces are:

- Ceramics;
- Glass;
- Metal (mainly aluminum and steel);
- Paper and Board;
- Plastics (in particular polyethylene, High-Density polyethylene, polyethylene terephthalate, polyvinyl chloride, polystyrene, and polycarbonate);
- Wood.

The Codex Alimentarius specifies that the primary requirement for packaging is to avoid being a source of contamination [4]. Established in 1963 by the Food and Agriculture Organization (FAO) and the World Health Organization, the Codex sets international standards, guidelines, and codes of practice for food safety, quality, and fairness in international food trade, encompassing 99% of the global population. Food packaging is pivotal in maintaining food quality and safety by acting as a protective shield against external contaminants, prolonging product shelf life, and preventing spoilage. However, packaging itself can introduce contaminants, jeopardising food safety and integrity. Contaminated food packaging poses health risks, foodborne illnesses, allergenic reactions, and economic consequences, such as costly recalls and reputational damage for producers [5, 6]. Preventive measures include stringent quality control throughout the production process, using high-quality packaging materials, adhering to good practices and standards, and implementing rigorous hygiene and health risk analyses, notably through the Hazard Analysis and Critical Control Points (HACCP) method. While not explicitly mandated by legislation, adopting the HACCP methodology is widely recognised and demanded by the national and international market to ensure food safety and prepare for certification schemes. HACCP facilitates both contamination prevention and compliance with industry standards, aligning with market expectations and enhancing consumer confidence in food safety [7].

## 1.3 Hygiene standards and consolidated decontamination techniques

A crucial yet often overlooked aspect is the biological contamination level of packaging materials, influenced by factors like handling frequency and exposure to air. Different materials exhibit varying propensities for microbial proliferation and require distinct sanitation methods. Production processes vary widely among packaging materials, presenting opportunities for both contamination and decontamination Metals, glass, and plastics manufacturing involve temperatures incompatible with microbial survival. Conversely, cellulosic materials present contamination risks due to source contamination, processing in humid environments conducive to microbial growth, and lower processing temperatures that sustain resilient microbial forms. Paper and board production may involve biocidal agents. Post-production contamination risks persist across all materials due to handling, non-sterile air drafts, insect presence, and machine contact. Acceptable hygienic conditions are indicated by cell counts below $10^4$ cells/cm$^2$, while values exceeding $10^7$ cells/cm$^2$ denote unsatisfactory conditions [8].

Microbial growth on packaging materials depends on factors like nutrient presence, moisture, and surface biofilm formation. Effective decontamination, typically targeting a 5-log reduction in microbial load, employs various thermal, chemical, and physical methods.

Traditional decontamination approaches include heat treatments, in particular dry heat (>180 °C), hot water and steam (130°C–150°C), and the utilisation of chemicals like hydrogen peroxide, ethylene or propylene oxides [9–11]. A growing interest is directed to more innovative methods like high-pressure processing, high-intensity pulsed electric field treatment, pulsed light, ozone-based treatments and cold-atmospheric plasma, which holds promise for diminishing harmful microorganisms in the context of the food industry more safely and sustainably [12–14].

## 1.4 Cold plasma technology

Plasma is the fourth state of matter, consisting of charged particles (ions and electrons) and neutral molecules. It can exploit several actions in many different applications, by tuning the different active agents that it includes [15]. These agents are the just-mentioned charged particles, chemically reactive species, such as free radicals, electromagnetic fields, radiations, and heat. For an application of interest, it is possible to control the operating conditions in order to maximise the presence of the active agents of interest. One main characteristic of plasmas (and the most relevant one to choose based on the application) is their macroscopic temperature. Plasmas can be consequently classified into:

- equilibrium (or thermal) plasmas, having the electron temperature in equilibrium with the heavy particles temperature, resulting in a macroscopic temperature higher than $10^4$ K;
- non-equilibrium (or cold plasmas), in which only the electron temperature is high, while the heavy particles–mainly influencing the gas temperature–keep values closer to ambient air.

Plasmas can also be classified on the basis of the pressure, which can be atmospheric or lower. Cold plasmas at atmospheric pressure will be hereinafter referred to as CAP (Cold Atmospheric Plasmas). Atmospheric pressure is often preferred in the industrial application perspective, since the arrangement is much simpler and economical. Furthermore, in-line continuous processes are economically sustainable only at atmospheric pressure, while low-pressure treatments should be carried out as batch processes.

The mechanisms of action related to the microbial inactivation are various. The active chemical components found in CAP demonstrate the ability to deactivate microorganisms on food surfaces due to their antimicrobial characteristics [14]. The primary effective components in the air plasma process include reactive oxygen species (ROS, including ozone, singlet oxygen, superoxide, peroxides, and hydroxyl radical) and reactive nitrogen species (RNS, mainly nitrogen oxides). The antimicrobial properties of these oxidative species can be attributed to lipid peroxidation within cell membranes and the oxidation of proteins and DNA within microbial cells [16]. Keener and Misra [17] have identified potential drivers for implementing this technology in the food industry, including lower energy requirements compared to current technologies, making it more environmentally friendly, reduced operational and maintenance costs due to simple systems with minimal upkeep and sanitation needs, improved chemical safety of foods through plasma inactivation and removal of pesticide and chemical residues, and its status as a green technology promoting environmental sustainability, as it only requires air and electricity to generate effective plasma.

Plasmas can be generated by a wide range of different sources, depending on the high voltage generator characteristics and the configuration of the source electrodes. The most used CAP sources are:

- Corona, in which a pointy electrode locally intensifies the electric field and allows the discharge;
- DBD (Dielectric Barrier Discharge), having two parallel (planar or concentric cylinders) electrodes separated by a dielectric barrier and a gap, in which the discharge takes place;
- Jets, both in the Corona or DBD configuration, presenting a gas flux which creates a plasma plume coming out from a nozzle;
- SDBD (Surface DBD), a particular configuration of DBDs with the ground electrode in the form of a mesh and without a gap, in order to generate plasma in the holes of the mesh.

# 2 Methods

This report was drafted following the PRISMA 2020 checklist, as described in the PRISMA statement and PRISMA explanation and elaboration [18–20].

A systematic research was carried out on the use of plasma systems for decontamination of surface packaging. The aim was to collect all studies that tested plasma's effectiveness on materials that could potentially or openly be traced back to packaging. The search focused on the years from 2000 to 2023, collecting all articles published in that period that contained terms concerning plasma, packaging materials and possible biological contaminants or decontamination-related terms in the title, abstract or keywords.

The search string used in the Scopus database is reported as Supplementary Material.

Reasons for exclusion from selection could be:

- publication language other than English;
- in-package treatments, i.e., where the plasma is generated inside a closed package;
- antimicrobial action carried out not directly on contaminated packaging, but, e.g., on food or test plates;
- low-pressure treatment of the material, as the analysis is addressed to atmospheric processes (however, studies in which plasma was produced at low pressure but the decontamination takes place at ambient pressure were included);
- removal of chemical contaminants as the analysis is addressed to the antimicrobial action of plasma);
- reviews.

The last search was done in December 2023. In addition to the study selected with this systematic search, other inherent papers in the authors' knowledge were included.

Different categories of information were chosen for the relevant data extraction from reports. First of all, the data about the plasma systems were collected, i.e., the type of plasma source and its dimensions or design characteristics, the operating conditions (voltage, current, frequency, power and power density, type of waveforms), the process gas and eventual other synergic agents such as water vapour, UV, etc. Then, contamination characteristics were summarised in the categories of pathogen strains, initial concentrations, and contamination procedure. Packaging sample material, geometries, and dimensions were reported as well. Finally, the fundamental data item is presented: the maximum inactivation achieved in terms of logarithmic reduction of the initial concentration.

The effect size of the studies, i.e., their outcome, can be presented differently. In this case, Standardised Mean Difference (*SMD*) was chosen as index. *SMD* is defined as difference in mean outcomes between groups, as follows:

$$SMD = Mean\,outcome\,of\,treated\,group - Mean\,outcome\,of\,control\,group$$

In the specific field of decontamination experiments, *SMD* could be identified as the logarithmic reduction of the pathogen charge (*LogR*), calculated by means of the following formula:

$$LogR = LogN_t - LogN_0$$

where $N_t$ represents the number of colonies in the treated group and $N_0$ in the control group.

Every individual study included in the quantitative analysis was associated with a *LogR* value for the outcome synthesis.

Averages of logarithmic reductions are also calculated for sub-groups of studies, by differentiating the pathogens in the main categories of: Gram-positive bacteria, Gram-negative bacteria, fungi, and viruses.

# 3 Results

## 3.1 Study selection

Subsequently, the flow diagram is presented (Figure 1). The records were first identified through Scopus database searching, as

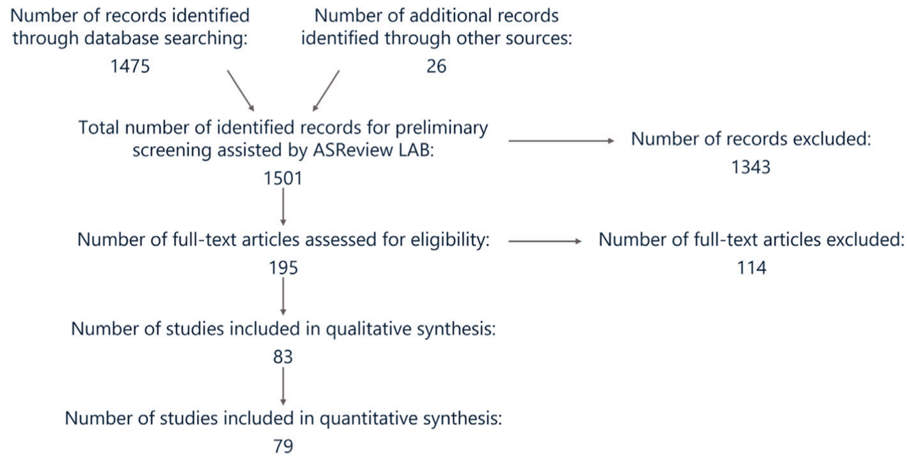


FIGURE 1
Flow diagram of records identification process.

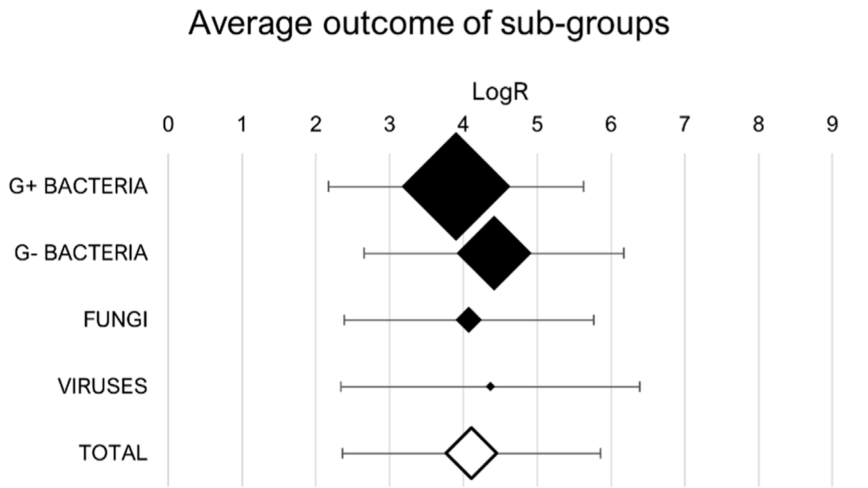


FIGURE 2
Synthesis of study outcomes: averages of sub-groups logarithmic reductions. Marker sizes represent the relative sub-group size.

previously illustrated. A few additional papers known by the authors were included as well. The collection of documents was then subjected to a preliminary screening on the software ASReview LAB (Zenodo, Switzerland), which assisted in the selection of eligible studies employing artificial intelligence tools. An additional amount was excluded by analysing the full-text articles for the above-mentioned reasons. A total number of 83 studies was included in the qualitative synthesis (i.e., the data extraction). Three papers did not present the data item considered to evaluate the outcome, i.e., the logarithmic reduction, and were consequently excluded from the quantitative analysis.

## 3.2 Summary of data and synthesis of results

The studies outcomes are summarised in the table reported as Supplementary Material. For each study included in the quantitative analysis, the outcome indicator, chosen as the logarithmic reduction of the contaminant concentration, is reported. The effect estimates of the individual studies are graphically synthesised in the forest plot, with standard deviation if specified. Each different combination of factors (plasma source, type of treatment, pathogen, packaging material) is considered individually and associated with its outcome. The total number of individual cases is 263. Only the most efficient case is considered when different operating conditions were investigated, such as different treatment times, distance between plasma and the substrate, gas flux or generator electrical settings. The averages of these results for groups of similar pathogens are also calculated and reported in Figure 2.

Observing the average reduction rates for the sub-groups, it is evident that Gram-positive bacteria are usually less sensitive than Gram-negative bacteria to plasma treatment since the mean logarithmic reduction is respectively 3.90 ± 1.73 and 4.42 ± 1.76. This difference is in accordance with the literature and due to their distinctive structure of cell membrane [21]. The anti-viral effect of plasma on contaminated packaging is a field that needs further investigation, since only 3 studies were included in the quantitative analysis. The mean efficacy against fungi, equal to 4.06 ± 1.76 LogR, reflects the total outcome relative to the whole research. In fact, the total outcome of the meta-analysis is an average of 4.11 ± 1.75 LogR over 256 individual cases, which leads to the conclusion that plasma treatments at atmospheric pressure are effective against foodborne pathogens.

### 3.2.1 Investigated pathogens

On the basis of the records collection and data extraction, it was possible to identify the most investigated pathogens in the field.

Gram-positive bacteria were the subject of study in 136 cases (51.3% of the total)[ [22–50] [51–74]]; in particular, 32 times the treatment was against endospores [40–42, 44, 46–49, 52, 54, 57, 58, 62, 70] and 5 times against biofilm of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* (single-species) [39] and *Streptococci* strains [44]. The most studied Gram-positive bacteria are:

- *Staphylococcus aureus* (50 occurrences, 8 times as *Methicillin-Resistant S. aureus*),
- *Bacillus subtilis* (16 occurrences),
- *Listeria monocytogenes* (11 occurrences),
- *Bacillus atrophaeus* (8 occurrences).

Gram-negative bacteria were the subject of study in 93 cases (35.1% of the total)[ [24, 25], [28–34], [36–38], [43–45], [48, 50, 51], [61–64], [69, 71] [75–93]]; in particular, 7 times in the form of biofilm of *Escherichia coli* (single-species) [78, 90, 94], *Pseudomonas aeruginosa* (single-species) [79, 83, 91] and *Salmonella* strains [88]. The most studied Gram-negative bacteria are:

- *Escherichia coli* (53 occurrences),
- *Staphylococcus typhimurium* (15 occurrences),
- *Pseudomonas aeruginosa* (8 occurrences),
- *Acinetobacter baumannii* (8 occurrences).

Fungi were investigated in 28 cases (10.6%) [23, 26, 31, 32, 45, 68, 73, 89, 95–99], 2 times as biofilm of *Candida albicans* [97, 100]. Among the total number of cases counted as “fungi,” thrice the contaminants were spores [26, 31, 57]. The most studied fungi are:

- *Candida albicans* (10 occurrences),
- *Aspergillus niger* (6 occurrences).

Only 6 experiments considered viruses [52, 62, 101–103]. It is meaningful that 4 out of 5 cited papers were published between 2021 and 2022, suggesting an increasing interest developed after the COVID-19 outbreak. This deduction is corroborated by the fact that the specific contaminant virus was a coronavirus in three studies [101–103]. The contaminant was a bacteriophage in the other two cases [52, 62].

### 3.2.2 Investigated packaging materials

Polymeric materials were treated in 58% of the cases. The most treated polymeric materials were:

- Polyethylene Terephthalate (36 cases),
- Polypropylene (34 cases),
- Polyethylene (24 cases.

Among the non-polymeric materials (occurred in 42% of the cases), very frequently used substrates were:

- Glass (52 cases),
- Stainless Steel (35 cases),
- Paper (12 cases).

### 3.2.3 Plasma sources

The following occurrences are referred to a total of 77 specified sources. The most frequently used are:

- DBD (29 times—38%),
- SDBD (18 times—23%),
- Jets (13 times—17%),
- Corona (7 times—9%).

In 3 out of the 84 selected studies, the plasma source was not specified at all, while it was just mentioned as a generic CAP in 5 articles. In 2 papers, 2 different sources were tested in the same paper.

### 3.2.4 Treatment methods

Different treatment methods are available when CAP are used. Their conventional classification is adopted in this systematic review:

- Direct treatment, in which plasma is in direct contact with the substrate,
- Indirect treatment, in which the anti-microbial action is exerted mainly by the reactive species, since plasma does not occupy the substrate area,
- Plasma-activated water or air, as media carrying the reactive species previously generated by plasma; air or water are subsequently conveyed in contact with the substrate.

Among the selected studies, direct treatment occurred 46 times (56.8%), indirect treatment 31 times (38.3%) and plasma-activated water/air 4 times (4.9%).

A final observation regards the possibility of exploiting synergies with other biocidal agents. Some attempts dealt with the combination of plasma and i) ultraviolet rays [23, 27, 31], ii) hydrogen peroxide [33, 96], iii) water in the form of water vapour [57], water spray [44] or water layer deposited onto the substrate [87].

## 4 Discussion and conclusion

According to the literature, plasma systems are proven effective in decontaminating packaging materials and food contact surfaces. The antimicrobial efficacy is demonstrated for various operating and environmental conditions, for different plasma source configurations and treatment methods from the direct treatment to the indirect one to the PAW (Plasma-Activated Water) or PPA

(Plasma-Processed Air) exposure. The plasma action inactivated a wide range of different microorganisms. The average inactivation was satisfactory according to European standards for the traditional chemical disinfectants BS EN 13697:2015+A1:2019 and BS EN 14885:2022 [104, 105], which state that the minimum LogR should be at least a 4 LogR for bacteria and at least a 3 LogR for fungi. In practical packaging industry scenarios, it will be crucial to evaluate the impact of initial microbial concentration on the plasma treatment efficacy. Notably, a study conducted by Fernández et al. [106] revealed an inverse relationship between initial concentration and treatment effectiveness since, for high concentrations, significant clumps, and multilayered structures form, potentially offering physical protection against CAP treatment.

In conclusion, future research should consider the proof-of-concept of the process and focus on optimisation, in particular, reducing treatment time and increasing effectiveness. An up-scaling is wished, considering the requirement of industrial facilities as well. In fact, the treatment time is often of the order of minutes, which is not compatible with typical industrial constraints. Furthermore, most of the plasma sources used for the purpose can treat only a limited portion of the surface, of the order of a few square centimeters. Only 3 studies [26, 30, 53] were able to conduct experiments as in-line processes, while all the others were performed statically. The transition from laboratory prototypes to industrial in-line systems should be the main aim of future research in the field.

## Author contributions

CM: Writing–original draft, Software, Resources, Methodology, Investigation, Data curation. MG: Writing–review and editing, Funding acquisition, Formal Analysis. RL: Visualization, Supervision, Project administration, Funding acquisition, Conceptualization, Writing–review and editing, Formal Analysis.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphy.2024.1399720/full#supplementary-material

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